

On each day, note daily signs in each animal and score using the following scale:

- 0 no signs
- 1 slight diarrhoea
- 2 marked diarrhoea (watery faeces)
- 3 dead

Calculate total scores for each animal over 10 days.

The test is invalid if fewer than 80 per cent of the animals given colostrum from the controls die or show severe signs of disease. The vaccine complies with the test if there is a significant reduction in score in the group of animals given colostrum from vaccinated dams compared with the group given colostrum from the unvaccinated controls.

### 2-3. MANUFACTURER'S TESTS

**2-3-1. Batch potency test.** It is not necessary to carry out the Potency test (section 3-4) for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. The following test may be used.

To obtain a valid assay, it may be necessary to carry out a test using several groups of animals, each receiving a different dose. For each dose required, carry out the test as follows. Use not fewer than 7 animals (for example rabbits, guinea-pigs, rats or mice) that do not have antibodies against the antigens stated on the label. Vaccinate not fewer than 5 animals, using one injection of a suitable dose. Maintain 2 animals as controls. Where the recommended schedule requires a booster injection to be given, a booster vaccination may also be given in this test provided it has been demonstrated that this will still provide a suitably sensitive test system. At a given interval within the range of 14-21 days after the last injection, collect blood from each animal and prepare serum samples. Use a suitable validated test such as an enzyme-linked immunosorbent assay (2.7.1) to measure the antibody response to each of the protective antigens stated on the label. The vaccine complies with the test if the antibody levels in the vaccinates are not significantly less than those obtained with a batch that has given satisfactory results in the test described under Potency and there is no significant increase in antibody titre in the controls.

Where animals that do not have antibodies against the antigens stated on the label are not available, seropositive animals may be used in the above test. During the development of a test with seropositive animals, particular care will be required during the validation of the test system to establish that the test is suitably sensitive and to specify acceptable pass, fail and retest criteria. It will be necessary to take into account the range of possible prevaccination titres and establish the acceptable minimum titre rise after vaccination in relation to these.

**2-3-2. Bacterial endotoxins.** A test for bacterial endotoxins (2.6.14) is carried out on the final lot or, where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or the mixture of bulk antigens immediately before addition of the adjuvant. The maximum acceptable amount of bacterial endotoxins is that found for a batch of vaccine that has been shown satisfactory in safety test 2-2-2-1 given under Choice of vaccine composition or in the safety test described under Tests, carried out using 10 animals. Where the latter test is used, note the maximum temperature increase for each animal; the vaccine complies with the test if the average temperature increase for all animals does

not exceed 1.5 °C. The method chosen for determining the amount of bacterial endotoxin present in the vaccine batch used in the safety test for determining the maximum acceptable level of endotoxins is used subsequently for testing of each batch.

### 3. BATCH TESTS

**3-1. Identification.** In animals that do not have antibodies against the antigens stated on the label, the vaccine stimulates the production of such antibodies.

**3-2. Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use* (0062).

**3-3. Safety.** Use 2 animals of one of the species for which the vaccine is intended and preferably, that do not have antibodies against the antigens stated on the label or, where justified, use animals with a low level of such antibodies as long as they have not been vaccinated against colibacillosis and administration of the vaccine does not cause an anamnestic response. Administer to each animal by a recommended route a double dose of the vaccine. Observe the animals at least daily for 14 days. Record body temperature before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily for 2 days.

The vaccine complies with the test if no animal shows notable signs of disease or dies from causes attributable to the vaccine; a transient temperature increase not exceeding 2 °C may occur.

**3-4. Potency.** The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-2-3) when administered by a recommended route and method.

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## NEWCASTLE DISEASE VACCINE (INACTIVATED)

### Vaccinum pseudopestis aviariae inactivatum

#### 1. DEFINITION

Newcastle disease vaccine (inactivated) (also known as avian paramyxovirus 1 vaccine (inactivated) for vaccines intended for some species) is a preparation of a suitable strain of Newcastle disease virus (avian paramyxovirus 1), inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for active immunisation of birds against Newcastle disease.

#### 2. PRODUCTION

##### 2-1. PREPARATION OF THE VACCINE

The vaccine virus is grown in embryonated hens' eggs or in cell cultures. The virus harvest is inactivated. The vaccine may be adjuvanted.

##### 2-2. SUBSTRATE FOR VIRUS PROPAGATION

**2-2-1. Embryonated hens' eggs.** If the vaccine virus is grown in embryonated hens' eggs, they are obtained from healthy flocks.

**2-2-2. Cell cultures.** If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines (5.2.4).



### 2.3. SEED LOTS

2.3-1. **Extraneous agents.** The master seed lot complies with the test for extraneous agents in seed lots (2.6.24). In these tests on the master seed lot, the organisms used are not more than 5 passages from the master seed lot at the start of the test.

### 2.4. CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for each species and category of birds for which it is intended. The following tests for Immunogenicity (section 2.3-1) may be used during the demonstration of efficacy.

2.4-1. **Immunogenicity.** A test is carried out for each route and method of administration to be recommended; the vaccine administered to each bird is of minimum potency. For chickens, the test for vaccines for use in chickens (section 2.4-1-1) is suitable for demonstrating immunogenicity. For other species of birds (for example, pigeons or turkeys), the test for vaccines for use in species other than the chicken (section 2.4-1-2) is suitable for demonstrating immunogenicity.

2.4-1-1. *Vaccines for use in chickens.* Use not fewer than 70 chickens, 21-28 days old, of the same origin and from a flock free from specified pathogens (SPF) (5.2.2). For vaccination, use not fewer than 3 groups, each of not fewer than 20 chickens. Choose a number of different volumes of the vaccine corresponding to the number of groups: for example, volumes equivalent to 1/25, 1/50 and 1/100 of a dose. Allocate a different volume to each vaccination group. Vaccinate each chicken by the intramuscular route with the volume of vaccine allocated to its group. Maintain not fewer than 10 chickens as controls. Challenge each chicken after 17-21 days by the intramuscular route with 6 log<sub>10</sub> embryo LD<sub>50</sub> of the Herts (Weybridge 33/56) strain of avian paramyxovirus 1. Observe the chickens at least daily for 21 days after challenge. At the end of the observation period, calculate the PD<sub>50</sub> by standard statistical methods from the number of chickens that survive in each vaccinated group without showing any signs of Newcastle disease during the 21 days. The test is invalid unless all the control birds die within 6 days of challenge. The vaccine complies with the test if the smallest dose stated on the label corresponds to not less than 50 PD<sub>50</sub> and the lower confidence limit is not less than 35 PD<sub>50</sub> per dose. If the lower confidence limit is less than 35 PD<sub>50</sub> per dose, repeat the test; the vaccine must be shown to contain not less than 50 PD<sub>50</sub> in the repeat test.

2.4-1-2. *Vaccines for use in species other than the chicken.* Use not fewer than 30 birds of the target species, of the same origin and of the same age, that do not have antibodies against avian paramyxovirus 1. Vaccinate in accordance with the recommendations for use not fewer than 20 birds. Maintain not fewer than 10 birds as controls. Challenge each bird after 4 weeks by the intramuscular route with a sufficient quantity of virulent avian paramyxovirus 1. The test is invalid if serum samples obtained at the time of the first vaccination show the presence of antibodies against avian paramyxovirus 1 in either vaccinates or controls, or if tests carried out at the time of challenge show such antibodies in controls. The test is invalid if fewer than 70 per cent of the control birds die or show serious signs of Newcastle disease. The vaccine complies with the test if not fewer than 90 per cent of the vaccinated birds survive and show no serious signs of avian paramyxovirus 1 infection.

### 2.5. MANUFACTURER'S TESTS

2.5-1. **Residual live virus.** The test is carried out in embryonated eggs or suitable cell cultures (5.2.4), whichever is the most sensitive for the vaccine strain. The quantity of

inactivated virus harvest used in the test is equivalent to not less than 10 doses of vaccine. The vaccine complies with the test if no live virus is detected.

2.5-2. **Batch potency test.** It is not necessary to carry out the Potency test (section 3-6) for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. The following tests may be used. Wherever possible, carry out the test for antigen content (section 2.5-2-1) together with the test for adjuvant (section 2.5-2-2).

*Vaccines for use in chickens.* The test for antigen content (section 2.5-2-1) together with the test for adjuvant (section 2.5-2-2) may be carried out; if the nature of the product does not allow valid results to be obtained with these tests, or if the vaccine does not comply, the test for serological assay (section 2.5-2-3) may be carried out. If the vaccine does not comply with the latter test, the test for vaccines for use in chickens (section 2.4-1-1) may be carried out. A test using fewer than 20 birds per group and a shorter observation period after challenge may be used if this has been shown to give a valid potency test.

*Vaccines for use in species other than the chicken.* Carry out a suitable test for which a satisfactory correlation has been established with the test for vaccines for use in species other than the chicken (section 2.4-1-2), the criteria for acceptance being set with reference to a batch that has given satisfactory results in the latter test. A test in chickens from an SPF flock (5.2.2) consisting of a measure of the serological response to graded amounts of vaccine (for example, 1/25, 1/50 and 1/100 of a dose with serum sampling 17-21 days later) may be used. Alternatively, the test for antigen content (section 2.5-2-1) together with the test for adjuvant (section 2.5-2-2) may be conducted if shown to provide a valid potency test.

2.5-2-1. **Antigen content.** The relative antigen content is determined by comparing the content of haemagglutinin-neuraminidase antigen per dose of vaccine with a haemagglutinin-neuraminidase antigen reference preparation, by enzyme-linked immunosorbent assay (2.7.1). For this comparison, *Newcastle disease virus reference antigen BRP*, *Newcastle disease virus control antigen BRP*, *Newcastle disease virus coating antibody BRP* and *Newcastle disease virus conjugated detection antibody BRP* are suitable. Before estimation, the antigen may be extracted from the emulsion using *isopropyl myristate R* or another suitable method. The vaccine complies with the test if the estimated antigen content is not significantly lower than that of a batch that has been found to be satisfactory with respect to Immunogenicity (section 2.4-1).

2.5-2-2. **Adjuvant.** If the immunochemical assay (section 2.5-2-1) is performed and if the vaccine is adjuvanted, the adjuvant is tested by suitable physical and chemical methods. For oil-adjuvanted vaccines, the adjuvant is tested in accordance with the monograph *Vaccines for veterinary use (0062)*. If the adjuvant cannot be adequately characterised, the antigen content determination cannot be used as the batch potency test.

2.5-2-3. **Serological assay.** Use not fewer than 15 chickens, 21-28 days old, of the same origin and from an SPF flock (5.2.2). Vaccinate by the intramuscular route not fewer than 10 chickens with a volume of the vaccine equivalent to 1/50 of a dose. Maintain not fewer than 5 chickens as controls. Collect serum samples from each chicken after 17-21 days. Measure the antibody levels in the sera by the haemagglutination-inhibition (HI) test using the technique



described below or an equivalent technique with the same numbers of haemagglutinating units and red blood cells. The test system used must include negative and positive control sera, the latter having an HI titre of  $5.0 \log_2$  to  $6.0 \log_2$ . The vaccine complies with the test if the mean HI titre of the vaccinated group is equal to or greater than  $4.0 \log_2$  and that of the unvaccinated group is  $2.0 \log_2$  or less. If the HI titres are not satisfactory, carry out the test for vaccines for use in chickens (section 2-4-1-1).

**Haemagglutination inhibition.** Inactivate the test sera by heating at  $56^\circ\text{C}$  for 30 min. Add 25  $\mu\text{l}$  of inactivated serum to the first row of wells in a microtitre plate. Add 25  $\mu\text{l}$  of a buffered 9 g/l solution of *sodium chloride R* at pH 7.2-7.4 to the rest of the wells. Prepare twofold dilutions of the sera across the plate. To each well add 25  $\mu\text{l}$  of a suspension containing 4 haemagglutinating units of inactivated Newcastle disease virus. Incubate the plate at  $4^\circ\text{C}$  for 1 h. Add 25  $\mu\text{l}$  of a 1 per cent V/V suspension of red blood cells collected from chickens that are 3-4 weeks old and free from antibodies against Newcastle disease virus. Incubate the plate at  $4^\circ\text{C}$  for 1 h. The HI titre is equal to the highest dilution that produces complete inhibition.

### 3. BATCH TESTS

**3-1. Identification.** When injected into animals that do not have antibodies against Newcastle disease virus, the vaccine stimulates the production of such antibodies.

**3-2. Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it comply with the test for sterility prescribed in the monograph *Vaccines for veterinary use* (0062).

**3-3. Extraneous agents.** Use 10 chickens, 14-28 days old, from an SPF flock (5.2.2). Vaccinate each chicken by a recommended route with a double dose of the vaccine. After 3 weeks, administer 1 dose by the same route. Collect serum samples from each chicken 2 weeks later and carry out tests for antibodies to the following agents by the methods prescribed in general chapter 5.2.2. *Chicken flocks free from specified pathogens for the production and quality control of vaccines:* avian encephalomyelitis virus, avian infectious bronchitis virus, avian leucosis viruses, egg-drop syndrome virus, avian bursal disease virus, avian infectious laryngotracheitis virus, influenza A virus, Marek's disease virus. The vaccine does not stimulate the formation of antibodies against these agents.

**3-4. Safety.** If the vaccine is intended for use in chickens, use 10 chickens, 14-28 days old, from an SPF flock (5.2.2). If the vaccine is not for use in chickens, use 10 birds of one of the species for which the vaccine is intended that do not have antibodies against Newcastle disease virus. Administer to each bird by a recommended route a double dose of the vaccine. Observe the birds at least daily for 21 days. The vaccine complies with the test if no bird shows notable signs of disease or dies from causes attributable to the vaccine.

**3-5. Residual live virus.** A test for residual live virus is carried out to confirm inactivation of Newcastle disease virus.

Inject  $2/5^{\text{th}}$  of a dose into the allantoic cavity of each of 10 embryonated hen eggs that are 9-11 days old and from SPF flocks (5.2.2) (SPF eggs), and incubate. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos and that from eggs containing dead embryos, excluding those dying within 24 h of the injection. Examine embryos that die within 24 h of injection for the presence of Newcastle disease virus: the vaccine does not comply with the test if Newcastle disease virus is found.

Inject into the allantoic cavity of each of 10 SPF eggs, 9-11 days old, 0.2 ml of the pooled allantoic fluid from the live embryos and, into each of 10 similar eggs, 0.2 ml of the pooled fluid from the dead embryos and incubate for 5-6 days. Test the allantoic fluid from each egg for the presence of haemagglutinins using chicken erythrocytes.

The vaccine complies with the test if there is no evidence of haemagglutinating activity and if not more than 20 per cent of the embryos die at either stage. If more than 20 per cent of the embryos die at one of the stages, repeat that stage; the vaccine complies with the test if there is no evidence of haemagglutinating activity and not more than 20 per cent of the embryos die at that stage.

Antibiotics may be used in the test to control extraneous bacterial infection.

**3-6. Potency.** The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-4-1) when administered by a recommended route and method.

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## NEWCASTLE DISEASE VACCINE (LIVE)

### Vaccinum pseudopestis aviariae vivum

#### 1. DEFINITION

Newcastle disease vaccine (live) is a preparation of a suitable strain of Newcastle disease virus (avian paramyxovirus 1). This monograph applies to vaccines intended for administration to chickens and/or other avian species for active immunisation.

#### 2. PRODUCTION

##### 2-1. PREPARATION OF THE VACCINE

The vaccine virus is grown in embryonated hens' eggs or in cell cultures.

##### 2-2. SUBSTRATE FOR VIRUS PROPAGATION

**2-2-1. Embryonated hens' eggs.** If the vaccine virus is grown in embryonated hens' eggs, they are obtained from flocks free from specified pathogens (SPF) (5.2.2).

**2-2-2. Cell cultures.** If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines (5.2.4).

##### 2-3. SEED LOTS

**2-3-1. Extraneous agents.** The master seed lot complies with the tests for extraneous agents in seed lots (2.6.24). In these tests on the master seed lot, the organisms used are not more than 5 passages from the master seed lot at the start of the tests.

##### 2-4. CHOICE OF VACCINE VIRUS

The vaccine virus shall be shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the birds for which it is intended.

The following tests for intracerebral pathogenicity index (section 2-4-1), amino-acid sequence (section 2-4-2), safety (section 2-4-3), increase in virulence (section 2-4-4) and immunogenicity (section 2-4-5) may be used during the demonstration of safety and immunogenicity.

**2-4-1. Intracerebral pathogenicity index.** Use vaccine virus at the least attenuated passage level that will be present in a batch of the vaccine. Inoculate the vaccine virus into the allantoic cavity of embryonated hens' eggs, 9- to 11- days-old, from an SPF flock (5.2.2). Incubate the inoculated eggs for